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ORIGINAL ARTICLE

A 7-day oral supplementation with branched-chain amino acids was ineffective to prevent muscle damage during a marathon

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Abstract The aim of this study was to determine the effectiveness of a 7-day oral supplementation with branched-chain amino acids (BCAA) to prevent muscle damage during a marathon. Forty-six experienced runners were randomly divided into two groups, one with BCAA supplementation ($n = 25$, supplemented with 5 g day^{-1} of powdered 1:0.5:0.5 leucine:isoleucine:valine, during the 7 days prior to the competition) and the other as a control group ($n = 21$, supplemented with an isocaloric placebo). Before the marathon race and within 3 min of finishing, leg muscle power was measured with a maximal countermovement jump and a urine sample was obtained. During the race, running pace was measured by means of a timechip. Myoglobin concentration was determined in the urine samples as an indirect marker of muscle damage. A visual analog scale (0–10 points) was used to assess leg muscle pain during the race. In the BCAA group, the mean running pace during the marathon was similar to the control group (3.3 ± 0.4 vs. 3.3 ± 0.5 m s^{-1}, respectively, 0.98). The pre- to post-race reduction in muscle power was similar in both BCAA and control groups (-23.0 ± 16.1 vs. -17.3 ± 13.8 %, $P = 0.13$). Post-race urine myoglobin concentration was similar in both BCAA and control groups (5.4 ± 7.5 vs. 4.5 ± 8.6 μg mL^{-1}, $P = 0.70$). Finally, there were no differences between groups in the perceived muscle pain during the race (6 ± 1 vs. 5 ± 1 points, $P = 0.80$). A 7-day supplementation of BCAA (5 g day^{-1}) did not increase the running performance during a marathon. Furthermore, BCAA supplementation was ineffective to prevent muscle power loss, muscle damage or perceived muscle pain during a marathon race.

Keywords Muscle damage · Running performance · Branched-chain amino acids · Nutritional supplementation · Myoglobinuria

Introduction

Marathon foot races (42.195 km) represent one of the most demanding endurance competitions because they greatly stress both cardiovascular and musculoskeletal systems for a long period of time (Maughan et al. 2007). Despite the severe physical demands of this competition, the attractiveness of the marathon has increased in the last few decades, mostly in the amateur running population that perceives the distance as a challenge. Completing a marathon involves both concentric and eccentric leg muscle actions repeated for 2–6 h, depending on the level of the runner. It is well established that marathon races produce severe damage to the runners' leg muscle fibers due to the high muscular demands of running for several hours (Schiff et al. 1978).

Marathon races are accompanied by microdamage to the sarcolemma, T-tubules, myofibrils and the cytoskeleton that persists for the 7 days after the competition (Hikida et al. 1983). As a result of the damage to the sarcolemma, several myocellular proteins are released into the blood stream and the increase of plasma concentrations of myoglobin (Smith et al. 2004), creatine kinase (Clarkson 2007) and LDH (Del Coso et al. 2013a) are typically used as indirect markers of muscle fiber damage. These

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myocellular proteins (mainly myoglobin) precipitate into the kidney and can lead to renal tubule obstruction and even renal failure, producing a syndrome known as exertional rhabdomyolysis (Clarkson and Eichner 2006). For this reason, muscle breakdown during the marathon has been investigated from a clinical perspective (Knochel 1990) while the relationship between muscle damage and running performance has been less well studied.

Exercise-induced muscle damage is typically manifested by a temporary decrease in strength and increased muscle soreness in the days following the competition (Howatson and van Someren 2008). However, recent investigations indicate that muscle fiber damage is also one of the most important causes for decreasing running performance during the marathon (Del Coso et al. 2013a, b). A strong correlation has been found between the urinary concentration of myoglobin and the loss of leg muscle power after a marathon (Del Coso et al. 2013b). In addition, the blood markers for muscle damage (myoglobin, creatine kinase and LDH) were higher in runners who drastically reduced their running pace during the marathon, in comparison to runners that kept a constant running pace during the race (Del Coso et al. 2013a). A similar relationship between exercise-induced muscle damage and performance has been found in the triathlon (Del Coso et al. 2012) suggesting that preventing muscle damage can be an effective strategy for increasing physical performance during endurance events.

Athletes have been experimenting with a variety of nutritional supplements to improve their sports performance (Ohtani et al. 2001). Among them, supplementation with branched-chain amino acids (BCAA; leucine:isoleucine:valine) has proven to be effective for reducing exercise-induced muscle damage although most investigations were focused on the recovery period subsequent to exercise (Nosaka et al. 2006; Shimomura et al. 2006; Howatson et al. 2012; Greer et al. 2007; Coombes and McNaughton 2000). Among them, leucine has been identified as the most effective BCAA to prevent exercise-induced muscle damage (Kirby et al. 2011). Only two investigations have been focused on the performance effects of BCAA during running. Koba et al. (2007) administered a drink containing 2.4 g of BCAA or a placebo to long distance runners during a 25-km run and they found that the post-run LDH concentration was lower in the BCAA trial. However, these authors did not measure running or muscle performance during the trials. Knechtle et al. (2011) administered an acute dose of 20 g of BCAA to one group of experienced ultra-endurance runners, and a placebo to another group of ultra-endurance runners just before and during a 100-km race. They found that running pace and plasma concentrations of myocellular proteins were not different between groups.

To our knowledge, no study has investigated the effects of a supplementation with BCAA on preventing muscle damage during the marathon. The aim of this investigation was to determine the effectiveness of a 7-day supplementation with BCAA to prevent muscle damage in the marathon. We hypothesized that BCAA supplementation would improve running pace during the race and would reduce the loss of muscle power after the race.

Methods

Subjects

A total of 50 amateur marathon runners, with previous experience in the marathon, participated in this investigation. The athletes were matched by age, training and running experience and then randomly assigned to the BCAA supplementation group or the control group. Table 1 presents the main morphological characteristics, personal best time and training status of the participants before the race. Participants had no previous history of cardiopulmonary diseases and they were not taking medications during the study. The participants were fully informed of the risks and discomforts associated with the experiments, and they gave their informed written consent to participate. The study was approved by the Camilo Jose Cela University Research Ethics Committee in accordance with the latest version of the Declaration of Helsinki.

Experimental protocol

A double-blind, placebo-controlled and randomized experimental design was used in this study. The athletes in

Table 1 Morphological characteristics, training status and race time of the participants

	BCAA ($n = 25$)	Control ($n = 25$)
Age (years)	41.9 ± 8.1	40.9 ± 6.8
Female runners (number)	3	4
Body mass (kg)	70.5 ± 9.8	71.6 ± 10.5
Body height (m)	1.71 ± 0.08	1.72 ± 0.07
Personal best time in marathon (min)	195 ± 18	195 ± 11
Training status	2.2 ± 0.5	2.5 ± 0.5

BCAA were a group of runners supplemented with branched-chain amino acids for 7 days and control was a group of runners supplemented with a placebo (cellulose)

Training status: 1 = from 0 to 35 km a week; 2 = from 36 to 70 km a week; 3 = from 70 to 105 km a week; 4 = more than 105 km a week, according to Smith et al. (2004)

the group with BCAA supplementation received, at least 1 week before the race, an unmarked opaque container with 35 g of pure BCAA (1:0.5:0.5 of leucine:isoleucine:valine) and a spoon labeled to provide a dose of 5 g. With the container, athletes received verbal and written instructions to ingest 5 g day^{-1} of powdered BCAA dissolved in 250 mL of tap water. The supplementation was consumed during the 7 days prior to the marathon race. Instructions indicated that BCAA supplementation should be consumed in the hours following their habitual training routines. The athletes in the control group received the same container, but filled with an isocaloric and isovolumetric placebo (1:1 cellulose:dextrose) and they were given the same instructions about beverage preparation and intake frequency. The consumption of the experimental substances (BCAA or placebo) the week before the race was certified by email reminders.

One to 3 days before the race, participants underwent a physical examination to ensure that they were in good health. Participants completed a short questionnaire on training status and medical history and filled out a form with their diet during the prior 7 days. Diet was analyzed using commercially available dietary analysis software (PCN 1.0, Cesnid, Spain). Subsequently, participants underwent a 5-min warm-up and were thoroughly familiarized with the jump test. Then, participants performed two maximal countermovement vertical jumps on a force platform (Quattrojump, Kistler, Switzerland). For this measurement, participants began stationary in an upright position with their weight evenly distributed over both feet. Each participant placed their hands on their waist to remove the influence of the arms on the jump, flexed their knees and jumped as high as possible while maintaining the hands on the waist. This measurement was performed with the competition clothes and shoes. Leg maximal power output during the jump was determined from ground reaction forces, as previously described (Del Coso et al. 2013b). The highest jump was used for analysis. After that, handgrip maximal force production in both hands was measured using a handgrip dynamometer (Grip-D, Takei, Japan). Finally, a sterile container was provided to the runners and verbal instructions were given to collect a urine specimen from the first-morning void the day of the race.

Participants were instructed to arrive at the start line 30 min before the onset of the race after their habitual warm-up routines. Participants brought the first-morning urine specimen and wore the same clothes that they would use during the race (T-shirt, shorts and competition shoes). Pre-race body mass was measured with a ±50 g scale (Radwag, Poland). Then, participants went to the start line to complete the race with no instructions about pace or drinking; therefore, no limitation was placed on the amount of fluid they could drink during the race and the participants ran at their own pace.

The marathon race was held in April 2012 in the area surrounding a city located at 655-m altitude (Madrid, Spain). The lowest altitude of the race was 600 m and the highest altitude was 720 m. The race was completed with a mean dry temperature of 27 ± 3 °C (range from 21 to 30 °C, temperature readings at 30-min intervals from 0- to 5-h after the race onset) and 27 ± 2 % relative humidity. During the race, participants wore a race bib with a timechip to calculate the actual amount of time that it took from the starting line of the race to the finish line (net time). Race time was also measured at 5-km intervals during the whole race.

Within 3 min after the race, participants went to a finish area and performed two countermovement vertical jumps and the handgrip maximal force test as previously described. Post-race body mass was recorded with the same scale and same clothes used for the pre-race measurement. Participants were instructed to avoid drinking until they were weighed and an experimenter was at the finish line to assure compliance Although post-race body mass measurement included the sweat trapped in the clothing, this represents an error lower than 10 % for the calculation of true hydration status (Cheuvront et al. 2002). The rate of perceived exertion (Borg scale; 6–20 points) and leg muscle soreness (0–10 points (Kirby et al. 2011)) was self-rated using visual analog scales. After that, subjects were provided with fluid (water and sports drinks) to promote urine production. Thirty to 60 min after the race, a representative sample of the post-race void was collected in a sterile container. After that, participants filled out a survey about the amount of fluid and food that they consumed during the race.

The urine samples obtained in the morning of the marathon and the urine samples obtained 60-min after the race were immediately analyzed (within 2 h) for specific gravity (Usg), pH, protein, glucose, ketones and bilirubin concentrations and the presence of leukocytes and erythrocytes using reactive strips (Combur Test, Roche, Switzerland), as previously described (Abian-Vicen et al. 2012). For these measurements, the strip was dipped in the urine sample and the excess was wiped off with a clean and absorbent paper. Then, the test strip was placed on the tray of a photometer (Urisys 1100, Roche Switzerland) and the aforementioned variables were measured after 1-min incubation. After each ten-sample batch, the photometer was calibrated with control strips provided by the manufacturer. A representative portion (5 mL) of the urine sample was frozen at −80 °C. At a later date, urine myoglobin concentration was determined by immunoluminiscence.

Statistical analysis

The Shapiro–Wilk test was used to check the normality of all variables. There were a similar number of male and female participants in each group (Table 1). After the first data analysis, it was found that both male and female participants responded similarly to the BCAA supplementation and their data have been merged and treated as a single group. Within-group (from pre- to post-race) and between-group (BCAA vs. placebo) comparison was performed using a two-way (time × group) ANOVA with repeated measures. After a significant F test (Geisser-Greenhouse correction for the assumption of sphericity), differences between means were identified using the Bonferroni adjustment. Differences in the variables measured once during the experimental protocol (e.g., training status, race time, diet content, etc.,) were identified with the Student's t test for independent samples. The intention-to-treat analysis (Gupta 2011) was used for the number of participants who finished the race in each group. All the data were analyzed with the statistical package SPSS v 19.0 (SPSS Inc., Chicago, IL). The significance level was set at $P < 0.05$. The results are presented as mean ± SD.

Results

Four athletes from the control group did not finish the marathon race, while all the participants in the BCAA group completed the race. The participants who did not finish the race reported muscular problems (muscle strains and muscle pain in upper and lower legs) that impeded the finalization of the race at their habitual running pace. The results of these four participants have been removed from the control group since they did not take part in the post-race measurements. Thus, the final number of participants was 25 in the BCAA group and 21 in the control group, and the pre- to post-race changes have been analyzed in these participants.

Baseline energy intake (1,922 ± 642 and 1,793 ± 411 kcal day^{-1}, for BCAA and control groups, respectively, $P = 0.25$), protein intake (108 ± 58 and 108 ± 50 g day^{-1}, $P = 0.50$) and branched-chain amino acids intake (16 ± 12 and 17 ± 11 g day^{-1}, $P = 0.44$) in the diet showed no differences between groups. The BCAA group ingested a supplementary 5 g day^{-1} of branched-chain amino acids as a part of the experimental design.

Figure 1 depicts the running speed for the BCAA and control groups during the marathon. The BCAA group ran at 3.5 ± 0.5 m s^{-1} during the first 5 km and their running speed progressively declined after the ½ marathon to 3.1 ± 0.5 m s^{-1} at the end of the race ($P < 0.05$). The same running pattern was observed in the control group (from 3.4 ± 0.5 to 3.1 ± 0.4 m s^{-1}; $P < 0.05$). There were no differences in the running pace between groups during the entire marathon ($P = 0.53$).

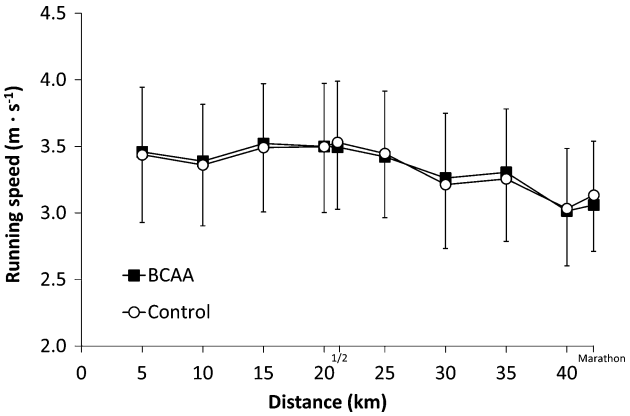

Fig. 1 Running pace during a marathon in a group of runners supplemented with branched-chain amino acids ($n = 25$; BCAA) for 7 days or a group of runners supplemented with a placebo ($n = 21$; control)

Table 2 Countermovement jump variables before and after a marathon race in a group of runners supplemented with branched-chain amino acids ($n = 25$; BCAA) for 7 days or a group of runners supplemented with a placebo ($n = 21$; control)

	BCAA	Control
Jump height (cm)		
Pre	27.1 ± 6.0	26.1 ± 5.4
Post	20.9 ± 6.7*	21.6 ± 5.7*
Change (%)	−23.0 ± 16.1	−17.3 ± 13.8
Leg muscle power (W kg^{-1})		
Pre	23.8 ± 3.1	22.3 ± 3.5
Post	18.9 ± 4.7*	18.4 ± 3.7*
Change (%)	−20.4 ± 15.9	−17.3 ± 11.4
Leg muscle force (N)		
Pre	1,266 ± 196	1,243 ± 220
Post	1,174 ± 211*	1,173 ± 215*
Change (%)	−7.3 ± 9.0	−5.7 ± 7.1
Leg muscle velocity (m s^{-1})		
Pre	1.3 ± 0.1	1.3 ± 0.1
Post	1.1 ± 0.2*	1.1 ± 0.1*
Change (%)	−17.7 ± 11.9	−13.5 ± 8.8

These data correspond only to the marathon finishers of each group

* Different from pre ($P < 0.05$)

Comparing with the pre-race values, height during the countermovement jump was reduced by 23.0 ± 16.1 % in the BCAA group (Table 2; $P < 0.05$) and 17.3 ± 13.8 % ($P < 0.05$) in the control group. However, there were no differences between groups for pre- to post-race change in the jump height ($P = 0.13$). Leg muscle power, force and velocity the jump were significantly reduced after the

Table 3 Urine responses before and after a marathon race in a group of runners supplemented with branched-chain amino acids ($n = 25$; BCAA) for 7 days or a group of runners supplemented with a placebo ($n = 21$; control)

	BCAA	Control
Urine specific gravity (units)		
Pre	1.018 ± 0.005	1.018 ± 0.005
Post	1.015 ± 0.007	1.017 ± 0.006
Myoglobin concentration (μg mL^{-1})		
Pre	0.0 ± 0.0	0.0 ± 0.0
Post	5.4 ± 7.5*	4.5 ± 8.6*
pH (units)		
Pre	5.9 ± 0.8	6.0 ± 1.2
Post	5.9 ± 1.5	5.7 ± 1.5*
Protein concentration (mg dL^{-1})		
Pre	2.4 ± 7.5	1.6 ± 6.3
Post	45.7 ± 55.7*	43.4 ± 46.6*
Erythrocyte concentration (U μL^{-1})		
Pre	0.0 ± 0.0	0.0 ± 0.0
Post	6.1 ± 12.2*	10.3 ± 9.1*
Leukocyte concentration (U μL^{-1})		
Pre	0.0 ± 0.0	0.0 ± 0.0
Post	10.9 ± 22.4*	47.4 ± 116.3*
Ketone concentration (mg dL^{-1})		
Pre	0.0 ± 0.0	0.0 ± 0.0
Post	3.0 ± 5.8*	2.1 ± 3.9*
Bilirubin concentration (mg dL^{-1})		
Pre	0.0 ± 0.0	0.0 ± 0.0
Post	0.4 ± 0.7*	0.5 ± 0.5*

These data correspond only to the marathon finishers of each group

* Different from pre ($P < 0.05$)

marathon (Table 2; $P < 0.05$) but the magnitude of the reductions was similar between groups ($P > 0.05$). In the BCAA group, handgrip force was reduced after the race in the dominant (from 425 ± 83 to 391 ± 85 N, $P < 0.05$) and non-dominant hands (from 408 ± 74 to 372 ± 77 N, $P < 0.05$). A similar reduction was obtained in the control group for the dominant (from 390 ± 84 to 383 ± 80 N, $P = 0.33$) and non-dominant hands (from 384 ± 74 to 358 ± 77 N, $P < 0.05$). After the race, the rate of perceived exertion (16 ± 2 vs. 15 ± 2 points for BCAA and control groups, respectively, $P = 0.26$) and the perceived muscle soreness (6 ± 1 vs. 5 ± 1 points, $P = 0.81$) were not different between groups.

From pre-race values (70.5 ± 9.8 and 71.7 ± 10.6 kg for BCAA and control groups, respectively), body mass was similarly reduced in both groups (3.0 ± 1.1 and 2.5 ± 1.1 %, $P = 0.13$). Pre-race urine specific gravity was 1.018 ± 0.005 for both groups and it remained unchanged after the race. While no myoglobin was detected in the pre-race urine specimens, this indirect marker of muscle damage increased after the race to 5.4 ± 7.5 μg mL^{-1} ($P < 0.05$) in the BCAA group and to 4.5 ± 8.6 μg mL^{-1} ($P < 0.05$) in the control group. However, the increase in urinary myoglobin was similar between groups ($P = 0.70$). Urinary pH values were unchanged from pre- to post-race in the BCAA group, while pH was significantly reduced in the urine of the control group after the race ($P < 0.05$). The marathon race produced significantly higher urinary concentrations of proteins, erythrocytes, ketones and bilirubin (Table 3; $P < 0.05$) but the magnitude of these increases was not modified by the supplementation with BCAA. Nevertheless, the increase in urinary leukocyte concentration tended to be higher in the control than in the BCAA group ($P = 0.06$).

Discussion

The aim of this investigation was to determine the effectiveness of a supplementation with 5 g day^{-1} of branched-chain amino acids (1.0:0.5:0.5 leucine:isoleucine:valine) for 7 days to increase physical performance and to prevent muscle breakdown during a marathon. For this purpose, we measured running and muscle performance in addition to physiological variables in two groups of amateur runners, one supplemented with BCAA and the other with an isocaloric placebo (control). The main outcomes were: (a) all participants supplemented with BCAA finished the marathon, while only 84 % of the participants in the control group completed the race; (b) in comparison to the control group, the athletes supplemented with BCAA did not improve running pace during the entire competition (Fig. 1) and they did not prevent the post-race reductions in leg muscle power and force during a countermovement jump (Table 2); (c) in comparison to the control group, the BCAA supplementation did not change the perceived effort or the perceived muscle soreness after the race; (d) the athletes supplemented with BCAA presented similar urinary myoglobin concentrations after the race than the athletes who were supplemented with a placebo. All these data suggest that BCAA supplementation was not ergogenic for amateur runners competing in a marathon.

Several investigations have been geared to determining the effects of BCAA supplementation as a strategy to treat exercise-induced muscle damage (Nosaka et al. 2006; Shimomura et al. 2006; Howatson et al. 2012; Greer et al. 2007; Coombes and McNaughton 2000). Coombes and McNaughton (2000) investigated the effects of 12 g day^{-1} of BCAA for 7 days on the muscle damage derived from 120-min of cycling, while BCAA supplementation was continued for 5 days after the exercise bout. In comparison

to a placebo, they found that serum concentrations of creatine kinase and LDH were decreased with BCAA supplementation. Howatson et al. (2012) investigated the effects of 20 g day^{-1} of BCAA for 7 days on muscle damage derived from 100 consecutive drop jumps, while BCAA supplementation was maintained for 5 days after the exercise routine. They found that BCAA supplementation improved muscle force recovery, while serum creatine kinase concentration and muscle soreness were reduced compared to a placebo. Shimomura et al. (2006, 2010) investigated the effects of an acute dose of 5 g of BCAA before 7 sets of 20 squats. They found that BCAA supplementation ameliorated the increase in serum myoglobin concentration and muscle soreness after exercise. All these investigation suggested that BCAA supplementation may improve the recovery of damaged muscles. However, the ergogenicity of BCAA intake to increase exercise performance seems unclear with the scientific data available to date. Based on these data, BCAA supplementation might be ergogenic in sport activities that induce muscle breakdown, such as prolonged running (Del Coso et al. 2013a).

A few studies have been devoted to determining the effectiveness of BCAA supplementation to improve physical performance during running. Blomstrand et al. (1991) provided 16 g of BCAA in four doses during a marathon race to a group of runners and a placebo substance to another group of runners. Overall, they found that runners who ingested BCAA did not improve their running performance. Similarly, Knechtle et al. (2011) determined the effects of a dose of 20 g of BCAA on ultra-endurance runners during a 100-km race. These authors found that BCAA did not change running pace or plasma concentrations of myocellular proteins (myoglobin and creatine kinase) which were not different between groups. On the other hand, Koba et al. (2007) investigated the effects of providing an acute dose of 2.4 g of BCAA to long distance runners during a 25-km run. They found that runners presented a lower serum LDH concentration after the race when they ingested BCAA. However, in that study there was no measurement of physical performance during the trials. Wisnik et al. (2011) provided male soccer players with 7 g of BCAA or a placebo before a multiple-choice reaction time during treadmill test and they found that BCAA administration shortened the time to complete the test by 10 %.

The current investigation presents some novelties compared with previous studies about BCAA supplementation and running performance. First, we have tested the effects of BCAA supplementation in both running and muscle performances. During the marathon race, running speed was measured by means of a time-chip at 5-km intervals. In the control group, 84 % (21 out of 25) of the participants finished the race while running pace was progressively reduced after the ½ marathon, as has been previously found in amateur runners (Ely et al. 2008). From the first 5 km, running pace was reduced by 9 ± 6 % at the end of the race. Interestingly, all the participants in the BCAA supplementation group (100 %, 25 out of 25) completed the race although they experienced a running speed reduction after the ½ marathon (Fig. 1). In the BCAA group, running pace was reduced by 11 ± 11 % from 5 to 42 km. In the Blomstrand et al. (1991) study mentioned above, authors subdivided participants in the marathon in faster (<185 min in the marathon) and slower (185–210 min) runners and they found that only slower marathoners benefited from BCAA ingestion. In our investigation, this subdivision of runners by race time did not change the main outcomes of the study. In addition, BCAA supplementation was ineffective to ameliorate the reduction in jump performance after the race (Table 2). Thus, we can safely conclude that 5 g day^{-1} of BCAA supplementation was not effective for increasing running or muscle performance in amateur runners during a marathon.

BCAA are abundant in muscle proteins and they account for 14–18 % of the total amino acids in muscle (Riazi et al. 2003). In contrast to other essential amino acids, BCAA are mainly catabolized in muscles and exercise increases muscle BCAA catabolism (Shimomura et al. 2010). Oral BCAA administration may promote protein synthesis by stimulating $_{m}$RNA translation (Garlick 2005) and suppress muscle protein breakdown during exercise (MacLean et al. 1994). To obtain these physiological effects, it is necessary that BCAA reach sarcoplasm of the active muscles. For this reason, a second novelty of this investigation is the use of a "loading" phase of BCAA for 7 days instead of a single dose, to increase the likelihood of BCAA reach intravascular (van Hall et al. 1995) and intramuscular spaces (MacLean et al. 1994). As previously mentioned, most studies that have found a preventive effect of BCAA on muscle recovery used a supplementation phase for several days. In our investigation, despite participants in the experimental group being supplemented with 5 g day^{-1} of powdered BCAA for 7 days, this intake was ineffective to prevent muscle damage or to increase running performance. Therefore, acute or chronic supplementations with BCAA were ineffective to increase physical performance during prolonged running. However, it is still possible that BCAA ingestion is effective to accelerate the muscle recovery process after a marathon.

In a recent publication (Del Coso et al. 2013b), it has been found that muscle power loss after the marathon was significantly correlated with post-race urine myoglobin concentration. This investigation showed that runners with greater levels of muscle breakdown were the ones with a reduced capacity to generate power during a

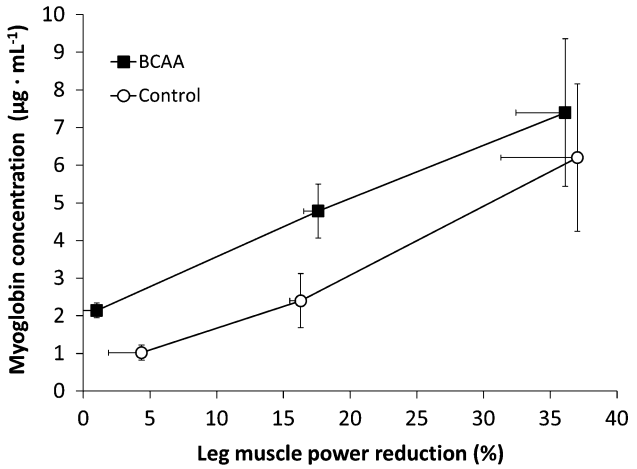

Fig. 2 Relationship between the urinary myoglobin concentration and the reduction in leg muscle power after a marathon race in a group of runners supplemented with branched-chain amino acids ($n = 25$; BCAA) for 7 days or a group of runners supplemented with a placebo ($n = 21$; control). Participants were grouped by their change in leg muscle power using 10 % intervals (0–10 %; 10–20 %; more than 20 %)

countermovement jump, suggesting that muscle fatigue in the marathon is associated with muscle fiber damage. Figure 2 depicts the relationship between muscle power reduction and urine myoglobin concentration in the participants supplemented with BCAA or with a placebo. Participants in each group (BCAA or control) were clustered by their change in leg muscle power using 10 % intervals (0–10 %; 10–20 %; more than 20 %), as previously suggested (Del Coso et al. 2013b). Again, leg muscle power loss during a countermovement jump and post-race myoglobinuria were positively correlated. This association was present in both groups of runners suggesting that BCAA supplementation does not prevent muscle damage or its consequences on muscle fatigue in the marathon.

After intense exercise, urine becomes more acidic due to an increase in ammonia excretion that leads to decreased urinary pH. This urine acidity occurs 10 min after ceasing exercise and it is maintained for up to 50–90 min afterwards (Wilson et al. 1924). Interestingly, we found that BCAA supplementation diminished the urine pH acidification produced during the marathon (Table 3). BCAA are used in patients with cirrhosis because this supplement may improve muscle and blood ammonia detoxification to glutamine although this effect is negligible in healthy people (Holecek 2013). In any case, the maintenance of urinary pH produced with the BCAA supplementation was equivalent to 0.2 ± 1.5 units and it was not related to any performance enhancement. Haematuria, proteinuria, leukocyturia and an increased presence of bilirubin and ketone bodies after the marathon were present in both groups. These urinary abnormalities are commonly found after intense running (McInnis et al. 1998) and they are the result of increased glomerular permeability due to decreased blood flow to the kidneys (Abian-Vicen et al. 2012). The urinary variables have also been related to increased damage to erythrocytes and leukocytes as a consequence of the compression of the capillaries produced by the muscle contractions during running (Kane and Cohen 2009). The supplementation with BCAA minimally affected these urinary indexes measured after the marathon, which in turn suggests the absence of effect of this supplementation on kidney filtration during exercise.

The present investigation possesses some relevant limitations that should be taken into account when applying the outcomes of this study to the runners' population. First, the experimental design included two groups of runners (experimental and placebo). Although the group assignation produced groups matched by age, training and running experience, it is still possible that the outcomes of this investigation are related to the individual differences. Another limitation is the assessment of muscle fiber damage using urine myoglobin concentration. Most investigations used serum concentration of intramuscular proteins to avoid the filtration effect of the kidneys on those proteins. Although it is indirect, we still consider that myoglobinuria is a valid measurement of muscle breakdown during a marathon. Finally, we used an ecologically valid context for this investigation (a real marathon competition) and some aspects of the investigation were not standardized (e.g., fluid and food ingestion during the race). Participants' reports and body mass changes indicated that both BCAA and control groups rehydrated similarly during the race; however it is still possible that these variables interfered with the outcomes of this investigation.

In summary, a 7-day supplementation with BCAA (5 g day^{-1}) was ineffective to increase the running performance during a marathon. Furthermore, BCAA supplementation was unsuccessful for preventing muscle power loss, muscle damage or perceived muscle pain during a marathon race. Thus, BCAA intake is not ergogenic for marathon runners, at least in the dosage used in this investigation.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Abian-Vicen J, Del Coso J, Gonzalez-Millan C, Salinero JJ, Abian P (2012) Analysis of dehydration and strength in elite badminton

players. PLoS One 7(5):e37821. doi:10.1371/journal.pone.0037821
Blomstrand E, Hassmen P, Ekblom B, Newsholme EA (1991) Administration of branched-chain amino acids during sustained exercise—effects on performance and on plasma concentration of some amino acids. Eur J Appl Physiol 63(2):83–88
Cheuvront SN, Haymes EM, Sawka MN (2002) Comparison of sweat loss estimates for women during prolonged high-intensity running. Med Sci Sports Exerc 34(8):1344–1350
Clarkson PM (2007) Exertional rhabdomyolysis and acute renal failure in marathon runners. Sports Med 37(4–5):361–363 (pii:37NaN22)
Clarkson PM, Eichner ER (2006) Exertional rhabdomyolysis: does elevated blood creatine kinase foretell renal failure? Curr Sports Med Rep 5(2):57–60
Coombes JS, McNaughton LR (2000) Effects of branched-chain amino acid supplementation on serum creatine kinase and lactate dehydrogenase after prolonged exercise. J Sports Med Phys Fit 40(3):240–246
Del Coso J, Fernandez D, Abian-Vicen J, Salinero JJ, Gonzalez-Millan C, Areces F, Ruiz D, Gallo C, Calleja-Gonzalez J, Perez-Gonzalez B (2013a) Running pace decrease during a marathon is positively related to blood markers of muscle damage. PLoS One 8(2):e57602. doi:10.1371/journal.pone.0057602
Del Coso J, Gonzalez-Millan C, Salinero JJ, Abian-Vicen J, Soriano L, Garde S, Perez-Gonzalez B (2012) Muscle damage and its relationship with muscle fatigue during a half-iron triathlon. PLoS One 7(8):e43280. doi:10.1371/journal.pone.0043280
Del Coso J, Salinero JJ, Abian-Vicen J, Gonzalez-Millan C, Garde S, Vega P, Perez-Gonzalez B (2013b) Influence of body mass loss and myoglobinuria on the development of muscle fatigue after a marathon in a warm environment. Appl Physiol Nutr Metab 38(3):286–291. doi:10.1139/apnm-2012-0241
Ely MR, Martin DE, Cheuvront SN, Montain SJ (2008) Effect of ambient temperature on marathon pacing is dependent on runner ability. Med Sci Sports Exerc 40(9):1675–1680. doi:10.1249/MSS.0b013e3181788da9
Garlick PJ (2005) The role of leucine in the regulation of protein metabolism. J Nutr 135(6 Suppl):1553S–1556S
Greer BK, Woodard JL, White JP, Arguello EM, Haymes EM (2007) Branched-chain amino acid supplementation and indicators of muscle damage after endurance exercise. Int J Sport Nutr Exerc Metab 17(6):595–607
Gupta SK (2011) Intention-to-treat concept: a review. Perspect Clin Res 2(3):109–112. doi:10.4103/2229-3485.83221
Hikida RS, Staron RS, Hagerman FC, Sherman WM, Costill DL (1983) Muscle fiber necrosis associated with human marathon runners. J Neurol Sci 59(2):185–203
Holecek M (2013) Branched-chain amino acids and ammonia metabolism in liver disease: therapeutic implications. Nutrition 29(10):1186–1191. doi:10.1016/j.nut.2013.01.022
Howatson G, Hoad M, Goodall S, Tallent J, Bell PG, French DN (2012) Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study. J Int Soc Sports Nutr 9(1):20. doi:10.1186/1550-2783-9-20
Howatson G, van Someren KA (2008) The prevention and treatment of exercise-induced muscle damage. Sports Med 38(6):483–503
Kane SF, Cohen MI (2009) Evaluation of the asymptomatic athlete with hepatic and urinalysis abnormalities. Curr Sports Med Rep 8(2):77–84. doi:10.1249/JSR.0b013e31819e0b8d
Kirby TJ, Triplett NT, Haines TL, Skinner JW, Fairbrother KR, McBride JM (2011) Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. Amino Acids 42(5):1987–1996. doi:10.1007/s00726-011-0928-9
Knechtle B, Knechtle P, Mrazek C, Senn O, Rosemann T, Imoberdorf R, Ballmer P (2011) No effect of short-term amino acid supplementation on variables related to skeletal muscle damage in 100 km ultra-runners—a randomized controlled trial. J Int Soc Sports Nutr 8:6. doi:10.1186/1550-2783-8-6
Knochel JP (1990) Catastrophic medical events with exhaustive exercise: "white collar rhabdomyolysis". Kidney Int 38(4):709–719
Koba T, Hamada K, Sakurai M, Matsumoto K, Hayase H, Imaizumi K, Tsujimoto H, Mitsuzono R (2007) Branched-chain amino acids supplementation attenuates the accumulation of blood lactate dehydrogenase during distance running. J Sports Med Phys Fit 47(3):316–322
MacLean DA, Graham TE, Saltin B (1994) Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. Am J Physiol 267(6 Pt 1):E1010–E1022
Maughan RJ, Watson P, Shirreffs SM (2007) Heat and cold: what does the environment do to the marathon runner? Sports Med 37(4–5):396–399 (pii:37NaN32)
McInnis MD, Newhouse IJ, von Duvillard SP, Thayer R (1998) The effect of exercise intensity on hematuria in healthy male runners. Eur J Appl Physiol 79(1):99–105
Nosaka K, Sacco P, Mawatari K (2006) Effects of amino acid supplementation on muscle soreness and damage. Int J Sport Nutr Exerc Metab 16(6):620–635
Ohtani M, Maruyama K, Suzuki S, Sugita M, Kobayashi K (2001) Changes in hematological parameters of athletes after receiving daily dose of a mixture of 12 amino acids for one month during the middle- and long-distance running training. Biosci Biotechnol Biochem 65(2):348–355
Riazi R, Wykes LJ, Ball RO, Pencharz PB (2003) The total branched-chain amino acid requirement in young healthy adult men determined by indicator amino acid oxidation by use of L-[1-13C]phenylalanine. J Nutr 133(5):1383–1389
Schiff HB, MacSearraigh ET, Kallmeyer JC (1978) Myoglobinuria, rhabdomyolysis and marathon running. Q J Med 47(188):463–472
Shimomura Y, Inaguma A, Watanabe S, Yamamoto Y, Muramatsu Y, Bajotto G, Sato J, Shimomura N, Kobayashi H, Mawatari K (2010) Branched-chain amino acid supplementation before squat exercise and delayed-onset muscle soreness. Int J Sport Nutr Exerc Metab 20(3):236–244
Shimomura Y, Yamamoto Y, Bajotto G, Sato J, Murakami T, Shimomura N, Kobayashi H, Mawatari K (2006) Nutraceutical effects of branched-chain amino acids on skeletal muscle. J Nutr 136(2):529S–532S (pii:136/2/529S)
Smith JE, Garbutt G, Lopes P, Pedoe DT (2004) Effects of prolonged strenuous exercise (marathon running) on biochemical and haematological markers used in the investigation of patients in the emergency department. Br J Sports Med 38(3):292–294
van Hall G, Raaymakers JS, Saris WH, Wagenmakers AJ (1995) Ingestion of branched-chain amino acids and tryptophan during sustained exercise in man: failure to affect performance. J Physiol 486(Pt 3):789–794
Wilson DW, Long W, Thompson H, Thurlow S (1924) Changes in the composition of the urine after muscular exercise. In: Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, NY). Royal Society of Medicine, pp 425–426
Wisnik P, Chmura J, Ziemba AW, Mikulski T, Nazar K (2011) The effect of branched chain amino acids on psychomotor performance during treadmill exercise of changing intensity simulating a soccer game. Appl Physiol Nutr Metab 36(6):856–862. doi:10.1139/h11-110